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ß-hydroxy-ß-methylbutyrate and its impact on skeletal muscle mass and physical function in clinical practice: a systematic review and meta-analysis

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Short running head: ß-hydroxy-ß-methylbutyrate and muscle mass

Abbreviations

ARG, Arginine

BIA, Bioelectrical Impedance Analysis

BUN, Blood Urea Nitrogen

CT, Computed Tomography

GLN, Glutamine

FFM, Fat free mass

HMB, β-hydroxy-β-methylbutyrate

ONS, Oral Nutrition Supplement

RCT, Randomized Controlled Trial

SIRS, Systemic Inflammatory Response Syndrome

SMD, Standard Mean Difference

3-MH, 3-methylhistidine

UUN, Urinary Urea Nitrogen

Abstract

Background

Loss of skeletal muscle mass and muscle weakness are common in a variety of clinical conditions with both wasting and weakness associated with an impairment of physical function. ß-hydroxy-ß-methylbutyrate (HMB) is a nutrition supplement that has been shown to favourably influence muscle protein turnover and thus has a potential role in ameliorating skeletal muscle wasting and weakness.

Objective

To investigate the efficacy of HMB alone, or supplements containing HMB, on skeletal muscle mass and physical function in a variety of clinical conditions characterized by loss in skeletal muscle mass and weakness.

Design

A systematic review and meta-analysis of randomized controlled trials reporting outcomes of muscle mass, strength and physical function was performed. Two reviewers independently performed screening, data extraction, and risk of bias assessment. Outcome data were synthesized through meta-analysis using a random-effects model and data presented as standardized mean differences (SMDs).

Results

Fifteen RCTs were included, involving 2137 patients. Meta-analysis revealed some evidence to support the effect of HMB alone, or supplements containing HMB, on increasing skeletal muscle mass (SMD = 0.25; 95% CI -0.00, 0.50; Z = 1.93; P = 0.05; I^2=58%) and strong evidence to support improving muscle strength (SMD =

0.31; 95% CI 0.12, 0.50; Z = 3.25; P = 0.001; I^2 =0%). Effect sizes were small. No effect on bodyweight (SMD = 0.16; 95% CI = -0.08, 0.41; Z = 1.34; P = 0.18; I^2 = 67%) or any other outcome was found. No study was considered to have low risk of bias in all categories.

Conclusion

HMB, and supplements containing HMB, increased muscle mass and strength in a variety of clinical conditions, although the effect size was small. Given the bias associated with many of the included studies, further high quality studies should be undertaken to enable interpretation and translation into clinical practice.

KEYWORDS: ß-hydroxy-ß-methylbutyrate, HMB, muscle, strength, nutrition, malnutrition, cancer cachexia, critical illness, sarcopenia

INTRODUCTION

Skeletal muscle wasting and weakness commonly occur with immobilisation and disuse (1), malnutrition (2), age-related sarcopenia (3), cancer cachexia (4) and during early critical illness (5). Furthermore, reduced muscle mass is associated with impaired physical function (6) and frailty (3), which drive morbidity (2, 7) and mortality (8-10). Interventions that can ameliorate, or even prevent, loss of muscle mass and improve physical function are a key clinical priority.

Depending on the technique used, skeletal muscle can be measured or estimated as either fat-free mass (FFM) or lean mass, although accuracy is variable (11). For example, bioelectrical impedance analysis (BIA) estimates FFM (sum of lean body mass and the bone mineral compartments) whereas DXA measures lean mass (body water, total body protein, carbohydrates, non-fat lipids, and soft tissue mineral) (11). Given the variety of techniques used to measure these body components, and that skeletal muscle is an important component of both fat free and lean mass, the term ‘muscle mass’ is used throughout this systematic review.

Muscle mass is maintained by a balance between muscle protein synthesis and muscle protein catabolism. Both resistance exercise and amino acid loading can enhance protein balance (12), however, resistance exercise is challenging, in particular during acute illness. This is compounded by insufficient protein intake in acute and chronic clinical patient populations (13-16) with anabolic resistance present in older patients (17, 18). For this reason, investigating novel interventions such as amino acids and their metabolites, which are not purely reliant on factors relating to appetite, are warranted.

β-hydroxy-β-methylbutyrate (HMB) is a metabolite of the amino acid, leucine, and its effects on skeletal muscle mass and strength has been investigated in athletes (19). HMB has several proposed mechanisms of action including stimulation of the mammalian target of rapamycin (mTOR) which leads to increased protein synthesis (20, 21) and attenuation of the proteasome pathways that lead to muscle protein catabolism (22, 23). Positive effects of HMB supplementation on maintenance of muscle mass in healthy older people undertaking bed rest, even in the absence of an exercise regimen, indicates this may be an efficacious nutrition intervention for immobile patients, such as during early critical illness (24). The effects of HMB supplementation have been studied in a variety of clinical conditions where muscle wasting is present, including critical illness (25), human immunodeficiency virus (HIV) (26) and cancer cachexia (27) with varying results.

The aim of the current study was to undertake a systematic review and meta-analysis to investigate the effects of HMB alone, and supplements containing HMB, on skeletal muscle mass and physical function in a variety of clinical conditions characterized by loss of muscle mass and skeletal muscle weakness.

MATERIALS & METHODS

This systematic review and meta-analysis was performed following guidelines from the Cochrane Handbook for Systematic Reviews of Interventions (28) and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (29). The protocol was pre-registered on PROSPERO (CRD42017058517).

Search strategy

A literature search for randomized controlled trials investigating the effect of HMB, and supplements containing HMB on muscle mass, strength or physical function in adult patients was conducted using electronic searching of four literature databases, two clinical trials databases, hand-searching of abstracts from three conference proceedings, back-searching of reference lists and discussions with key opinion leaders. One investigator (DEB) performed a database search (last search date 25 September 2018) using MEDLINE, Web of Science, EMBASE and CINAHL using a pre-determined search strategy (**Supplemental Methods**). Limits were applied to the electronic search, restricting studies to those including adults and humans, and published in the English language only. No date range restrictions were applied. The international trial databases Clinical Trials (www.clinicaltrials.gov) and ISRCTN (www.ISRCTN.com), were also searched for completed, unpublished studies. Abstracts of the following conference proceedings were hand-searched to identify any potentially relevant studies: American Society for Parenteral and Enteral Nutrition, European Society of Clinical Nutrition and Metabolism, and the Society of Sarcopenia, Cachexia and Wasting Disorders. Reference lists of relevant studies and previous systematic reviews were manually reviewed for additional studies not identified in the electronic search.

Study selection

After removing duplicates and non-relevant material, titles and abstracts identified from the search were screened independently by two investigators (DEB, AL). Potentially eligible studies had their full texts screened for eligibility by the same two investigators (DEB, AL). Inclusion and exclusion criteria are shown in **Table 1**.

Data extraction was performed independently by two investigators (DEB, AL) using a pre-specified data collection form. Data were cross-checked for discrepancies and

corrected where appropriate. The data extracted included author details, year of publication, participant characteristics, details of intervention and control and results relating to the aim of the systematic review. Authors of the included studies were contacted to obtain missing data where required.

Assessment of risk of bias

Risk of bias was assessed independently by two investigators (DEB, AL) according to the Cochrane Collaboration Risk of Bias Tool (28). Studies were assessed at both the individual and study level and included the methods used to generate randomization, conceal allocation, blind participants and personnel along with assessing incomplete outcome data, selective reporting and other sources of bias.

Where disagreements between the two researchers (DEB, AL) on study eligibility, data extraction and risk of bias assessment were not resolved by consensus, a third investigator was available to arbitrate (LW).

Data synthesis and statistical analysis

The primary outcome of interest in this systematic review was the change in skeletal muscle mass. Secondary outcomes included the effect on body composition, strength, physical function and surrogate markers of muscle wasting.

Meta-analyses were performed on the extracted data, where appropriate, using a random effects model in Review Manager 5.3 (RevMan5, Copenhagen, Denmark). Where studies included multiple time points, data from the end of the intervention was used in the overall meta-analysis. However, sub-analyses were also performed on outcome data measured in four-week epochs (0-4 weeks, 4-8 weeks, 8-12 weeks, >12 weeks) in order to determine any effects of the duration of the intervention on outcome. For studies using multiple methods to measure muscle

mass or strength (e.g. both BIA and air displacement plethysmography or handgrip and leg extensor strength), data from the most frequently used method across the studies was used in the overall meta-analyses. However, sub-analyses were also performed on each method of measuring the outcome where adequate (≥3) studies were available.

Given the varying methods used to measure muscle mass and strength, standard mean difference (SMD) with 95% confidence intervals (CI) were used to express effect size estimates. SMD values of 0.2, 0.5 and 0.8 were defined as small, moderate and large effect sizes respectively (30). One study did not present results for the overall group and therefore the results of the two subgroups were entered as independent groups in the meta-analyses (31).

As the reporting of outcome data varied across studies (absolute values at end of intervention, change scores), change scores were calculated from the available data and standard deviations imputed according to the Cochrane Handbook for Systematic Reviews of Interventions (28). Where two standard deviations were calculated, we imputed the largest to reduce the bias associated with this method.

Where change data were presented, but without mean and standard deviation or standard error, SMD was calculated from the reported p value. Studies reporting only median and interquartile range were converted to mean and standard deviations using a referenced formula (32). If more than half of studies included in a meta-analysis required imputation of standard deviations, rather than imputing change scores, the value at the end of the intervention period was used for that outcome. The meta-analysis for muscle strength was the only outcome where this was done. Sub-group analysis was undertaken where there were enough studies to explore the

effect of HMB presentation (e.g. alone, in ONS or combined with amino acids) on overall outcome.

Heterogeneity of results between studies was determined by I^2, with values of 25-49.9% considered low, 50-74.9% considered moderate and 75-100% considered high heterogeneity. Test for overall effect (Z score) was regarded significant at $P \leq 0.05$. Effect sizes were also relied upon as these are of greater clinical relevance and recommended by both the American Statistical Association (33) and the Cochrane Handbook for Systematic Reviews of Interventions (28). Funnel plots were generated to assess for evidence of asymmetry and possible publication bias or effects due to the small size of some studies (34).

RESULTS

Study Selection

A total of 1426 results were generated from the search strategy (**Figure 1**). None were obtained from discussions with experts in the field or back-searching reference lists and hand-searching conference abstracts. After duplicates were removed, 840 records were available for title and abstract screening. Of these, 21 records were retrieved for full-text screening with 15 RCTs being eligible for inclusion.

Study characteristics

The 15 eligible RCTs involved a total of 2137 adults (25-27, 31, 35-45). Authors of seven studies were contacted to provide additional data, of whom only one provided the requested data. All studies included patients who commonly experience muscle wasting as part of their clinical condition, including older care home residents

receiving tube feeding (n=1) (36), hospitalised older people with malnutrition / sarcopenia (n=2) (31, 43), hospitalised older people undergoing orthopaedic intervention (n=3) (38, 42, 44), critically ill (n=2) (25, 35), cancer cachexia (n=2) (27, 41), HIV (n=1) (26), maintenance haemodialysis (n=1) (39), rheumatoid cachexia (n=1) (40), gastric bypass (n=1) (45) and bronchiectasis (n=1) (37). Study characteristics and intervention data are reported in **Table 2.**

Three of the studies used HMB as a single supplement (35, 36, 39), seven used HMB in combination with arginine and glutamine (HMB/ARG/GLN) (25-27, 38, 40, 41, 45) and five used a high protein oral supplement containing HMB and other nutrients (31, 37, 42-44) (Table 2). All but one study (35) provided a dose of 3 g/d HMB. There was considerable variability in the duration of the intervention, ranging from seven days to six months (Table 2).

Risk of bias

No study was considered low risk of bias in all categories. Blinding of participants and personnel was uncommon, whilst selection (random sequence generation) and attrition bias had the lowest overall risk of bias (**Figure 2**).

Skeletal muscle mass

Ten of the 15 included studies reported measures of skeletal muscle mass (either FFM or lean mass) (26, 27, 31, 37-41, 44, 45) using DXA (31, 37, 39, 40, 45), air displacement plethysmography (Bod Pod) (26, 27), BIA (27, 37, 41, 44) or computed tomography (CT) (38) with measurement periods ranging from 4 weeks to 6 months (Table 2).

Meta-analysis was possible for 9 of these 10 studies (26, 27, 31, 37-40, 44, 45). Change scores were calculated from the available data and standard deviations imputed for four studies (37-39, 44). Median and interquartile range were converted to mean and standard deviation in one study (31). Some evidence was found to support supplementation with HMB alone, or supplements containing HMB, on increasing skeletal muscle mass compared with control, but the effect size was small (SMD = 0.25; 95% CI -0.00, 0.50; Z = 1.93; P = 0.05) (**Figure 3**). Moderate heterogeneity was present between studies (I^2 = 58%, p = 0.01).

Sub-group analyses according to the type of supplementation (HMB alone, combined with arginine and glutamine or within an ONS) revealed some evidence to support an increase in muscle mass when HMB/ARG/GLN was used, with a moderate effect size (SMD = 0.49, 95% CI -0.01, 0.99, Z = 1.93; P = 0.05; I^2 67%, p=0.02. (Figure 3).

Four studies included muscle mass measurements at multiple time points (27, 31, 37, 45). Therefore, sub-group analysis was performed according to duration of the intervention. No evidence was found to support improvements in muscle mass when the intervention was provided less than 4 weeks (27, 44, 45) (SMD = 0.34, 95% CI -0.21, 0.90, Z = 1.22; P = 0.22; I^2 = 69%, p=0.04), 4-8 weeks (26, 38, 45) (SMD = 0.60, 95% CI -0.06, 1.25; Z = 1.79, P= 0.07; I^2 =59%, p=0.09), 8-12 weeks (31, 37, 40) (SMD = 0.16, 95% CI -0.28, 0.59, Z = 0.71, P = 0.48, I^2 = 76%, p=0.005) and greater than 12 weeks (27, 31, 37, 39) (SMD = 0.08, 95% CI = -0.12, 0.28, Z = 0.79, P = 0.43; I^2 = 0%, p=0.62).

The study by Berk et al (41) was excluded from meta-analysis as only percent change in muscle mass was reported and absolute change could not be determined

231 from the data reported. This study showed no difference in muscle mass in cancer
232 cachexia following 8-weeks supplementation with HMB/ARG/GLN.

233 **Body weight and composition**

234 Thirteen studies measured body weight (26, 27, 31, 35-38, 40-45), six studies
235 reported fat mass (27, 31, 37, 39, 40, 44), three studies reported mid-arm muscle
236 circumference (36, 37, 42) three reported triceps skin fold (TSF) (26, 36, 41) and two
237 reported thorax, calf, waist and hip circumference (36, 42).

238 Meta-analysis for change in bodyweight could be performed for 12 of the 13 studies
239 (26, 27, 31, 35-38, 40, 42-45). One was excluded as only percent change in
240 bodyweight was reported (41). Four of the included studies had change scores
241 calculated from the available data and standard deviations imputed (37-39, 44).
242 Median and interquartile range were converted to mean and standard deviation in
243 one study (31). There was no evidence to support the effect of HMB, or supplements
244 containing HMB on change in bodyweight in the overall meta-analysis (SMD = 0.16;
245 95% CI = -0.08, 0.41; Z = 1.34; P = 0.18) or in sub-group analysis according to
246 supplement type (**Figure 4**). Heterogeneity was moderate (I^2 = 67%, p=0.0003).

247 All six studies reporting fat mass were included for meta-analysis (27, 31, 37, 39, 40,
248 44). Four of the included studies had change scores calculated from the available
249 data and standard deviations imputed (37-39, 44) and median and interquartile
250 range were converted to mean and standard deviation in one study (31). Overall,
251 there was no evidence to support a change in fat mass between patients receiving
252 HMB and controls (SMD = 0.03; 95% CI -0.27, 0.34; Z = 0.21; P=0.83; I^2 = 58%;
253 p=0.03). Sub-group analysis was not undertaken due to the small number of studies.

Studies reporting other measures of body composition were unsuitable for meta-analysis. No difference in TSF was reported in three studies (36, 41, 42). Measures of arm or body area circumference were reported in three studies (36, 37, 42), however only Hsieh et al (36) reported a greater increase in waist circumference after 14 days of HMB alone vs. control (0.97 ±4.46% vs -0.89 ±4.45%, p=0.026), which continued to day 28 of supplementation (2.24 ±4.64% vs -3.42 ±4.45%, p<0.05) with additional gains in calf circumference at this time point (2.57 ±5.02% vs -3.63±4.24%, p<0.05). Baseline difference in BMI were controlled for in this study.

Muscle strength

Measures of strength were reported in seven studies (31, 37-40, 42, 44), specifically isokinetic knee extensor and elbow flexor strength and handgrip strength. Absolute strength at the end of the intervention period was used for this meta-analysis. Six studies (37-40, 42, 44) were included in the meta-analysis revealing strong evidence that HMB or supplements containing HMB improved muscle strength compared with controls, but with a small to moderate effect size (SMD = 0.31; 95% CI 0.12, 0.50; Z = 3.25; P = 0.001; I^2 =0%) (**Figure 5**). Sub-group analysis according to supplement type demonstrated strong evidence to support the use of HMB alone (SMD = 0.26, 95% CI -0.00, 0.53, Z = 1.95, P = 0.05) and ONS containing HMB (SMD = 0.37; 95% CI 0.06, 0.68; Z=2.31; P=0.02; I^2 = 0%) on improving muscle strength (Figure 6), although there was only one study providing HMB alone. Effect sizes were small to moderate.

Three studies measured handgrip strength only (37, 42, 44), two studies measured leg strength only (38, 39), one study measured both handgrip and leg strength (31) and one study measured handgrip, leg and elbow strength (40). There was strong evidence to support an increase in handgrip strength (SMD = 0.38, 95% CI 0.10,

279 0.66; Z = 2.63, P = 0.008; I^2 = 0%, p=0.75) **(Supplemental Figure 1)** and leg
280 extensor strength (SMD = 0.28, 95% CI 0.08, 0.48, Z = 2.73, P= 0.006; I_2 = 0%,
281 p=0.85) **(Supplemental Figure 2)** with the intervention. However, effect sizes were
282 small to moderate.

283 The study by Cramer et al (31) was excluded from this meta-analysis as only data on
284 change in handgrip and leg strength are provided. Leg strength was measured at 12
285 and 24 weeks. There were no differences found between treatment groups for leg
286 strength in the overall study population, but participants classified as having
287 sarcopenia and normal grip strength displayed significantly greater increases in leg
288 strength in the intervention compared with control group (p=0.032).

289 **Physical Function**

290 Four studies reported measures of functional ability (31, 39, 40, 44), including gait
291 speed (31, 39, 44), sit-to-stand (39, 40), shuttle walk (39) and the 8 foot up-and-go
292 test (39). Meta-analysis could not be performed due to the nature of data reporting.
293 None of the four studies reported between-group differences in any outcome of
294 physical function, however two studies reported no significant changes in physical
295 function in the HMB group (39, 44) and two studies reported within-group
296 improvements in physical function over time in the HMB group (31, 40).

297 **Surrogate markers of muscle wasting**

298 Six studies reported surrogate markers of muscle wasting including blood urea
299 nitrogen (BUN) (26, 27, 35, 36, 39), urinary urea nitrogen (UUN) (36), nitrogen
300 balance and 3-methylhistidine (3-MH) excretion (25). Meta-analysis was not possible
301 due to the reporting of data or an insufficient number of studies to include in the
302 analysis.

Although BUN and UUN were reported in several studies, only two specifically investigated these as a surrogate marker of muscle wasting (35, 36) whilst the others measured these to investigate the safety of taking either HMB or a mixed amino acid supplement containing HMB (HMB/ARG/GLN) (26, 27, 39). Results from the studies were inconsistent. Only one study found a significant decrease in BUN in the intervention group at day 14, but not day 28 of the study period (36). In contrast, May et al (27) report a significant increase in BUN in the group receiving HMB/ARG/GLN compared with a decrease in the control which was significantly different between the two groups ($P<0.05$). Clark et al (26) also report an increase in BUN in the HMB/ARG/GLN group, but overall results were not displayed. Lastly, Fitschen et al (39), report that BUN differences between the groups were not significant.

In the one study reporting UUN as a surrogate measure of muscle wasting, the change in 24-hour UUN excretion was significantly lower in the group receiving HMB compared with control participants at both 14 days (-12.5% vs 29.7%, $p=0.02$) and 28 days (-30.7% vs 15.7%, $p<0.001$) (36). Kuhls et al (25) reported no difference in UUN excretion between the three groups (HMB alone, HMB/ARG/GLN or placebo), but nitrogen balance was significantly improved following HMB alone compared with the HMB/ARG/GLN from day 1 to day 7 and from day 8 to day 14 ($p<0.05$). The control group were also in greater negative nitrogen balance compared to the HMB alone, but this was not significant ($p<0.08$). Muscle proteolysis as measured by 3-MH was not different between the three groups.

Other clinical outcomes

Other clinical outcomes included inflammation (35) or infections (SIRS score) (25), degree of sepsis (25), mortality (43), hospital and ICU length of stay (25, 43) and hospital readmissions (43) and vitamin D status (31, 43, 44).

Kuhls et al (25), found a decreased incidence of patients with SIRS score 3 or 4 on days 3 and 7 in the HMB group, but no difference in other clinical outcomes. The use of an ONS containing HMB compared to placebo reduced 90-day mortality in one study (4.8% vs. 9.7%, p=0.018), but this was a secondary outcome (43). No other differences were reported as significant for the remaining clinical outcomes.

Adverse events

Five studies reported absolute numbers or percentages of adverse events (31, 36, 40, 41, 43). Marcora et al (40), reported significantly lower proportion of participants with gastrointestinal discomfort in those receiving HMB/ARG/GLN compared with placebo (28% vs. 67% p = 0.02). Hsieh et al (36), reported that some patients dropped out due to the development of scabs, but numbers and groups were unclear. The other three studies reported similar numbers of adverse events between groups.

Publication bias

Visual inspection of the funnel plots for all outcomes did not reveal substantial asymmetry and therefore publication bias (**Supplemental Figures 3-5**). Statistical tests to explore funnel plot asymmetry were not undertaken due to the use of SMD in the meta-analysis as per the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (28).

DISCUSSION

This systematic review and meta-analysis aimed to investigate the effects of HMB, and supplements containing HMB, on skeletal muscle mass and physical function in a variety of clinical conditions characterized by loss of muscle mass and skeletal

muscle weakness, including ageing and critical illness. We found some evidence to support a positive effect of HMB on the change in muscle mass and strong evidence to support an increase in absolute muscle strength. However, the effect size was small in both instances. Sub-group analysis indicated that participants receiving HMB and supplements containing HMB were significantly stronger compared with the control group, but with only a small to moderate effect size. These findings may have important clinical implications given the well-documented detrimental effects of low muscle mass and skeletal muscle wasting in a number of clinical conditions (6-10). Of major clinical relevance, is that HMB alone, and supplements containing HMB, have a strong safety profile without increased incidence of adverse events compared to placebo or standard care groups used across this range of clinical conditions.

To our knowledge, this is the first meta-analysis investigating the effect of HMB on strength in a variety of clinical conditions. As it is common for studies to use multiple measurements for each outcome, we also performed separate meta-analysis for the outcomes of handgrip strength and leg extensor strength and observed that evidence in support of this remained strong providing further support for the use of HMB and supplements containing HMB.

Of interest, none of the studies included in the meta-analysis for strength reported a between group difference in absolute muscle mass following the intervention. This is perhaps unsurprising given the complex relationship between nutritional status and muscle mass, muscle quality, muscle strength and physical function that is non-linear. Indeed, an improvement in muscle quantity may not translate to a proportional improvement in volitional, or non-volitional, force production (46), and in turn a commensurate change in performance of physical function, which additionally

requires coordination of cognitive and executive function. Furthermore, establishing a true baseline of physical functional status is challenging in the absence of robust markers to determine pre-morbid ability. Careful consideration should be given to selection of these outcomes in such trials (47).

Despite including a potentially heterogeneous group of adult patients, the results of the current study provide some evidence to support the beneficial effect of HMB on muscle mass and strong evidence to support the effect on strength. This is in line with previous systematic reviews that have reported the effect of HMB in older people (48) and athletes (19). By only including studies in adults with a clinical condition associated with skeletal muscle wasting, the current systematic review and meta-analysis is unique and of major clinical importance as it highlights the potential of using HMB, and supplements containing HMB, in the management of a variety of clinical conditions characterised by skeletal muscle wasting and weakness. Interestingly, the increase in muscle mass reported in this current study was observed without changes in either body weight or FFM, although this may be more reflective of methodological differences in the included studies rather than any mechanistic effect of HMB itself and should be interpreted with caution.

Many conditions, such as critical illness and cancer cachexia, result in reduced muscle mass (4, 5), strength and physical function (2, 3, 6). Whilst the shared common pathway is an imbalance between muscle protein synthesis and muscle protein breakdown, the specific intra-cellular proximal signalling pathways leading to this differ between conditions (5, 49). Older patients also display anabolic resistance whereby muscle protein synthesis is resistant to stimuli such as resistance training and amino acid loading (17, 18), a serious clinical consideration with an increasing ageing population. Moreover, concurrent inflammation (50) and immobilisation, a

hallmark of critical illness (1, 51) and other acute clinical conditions, could further contribute to skeletal muscle wasting. Interventions that target muscle protein turnover, in terms of reducing muscle protein breakdown and enhancing muscle protein synthesis as well as reducing inflammation (52), such as HMB, are of increasing clinical interest.

Interventions varied in the studies in this systematic review and included HMB alone, HMB in combination with the amino acids arginine and glutamine and HMB in ONS with high protein and vitamin D. However, there was relative consistency in the doses used across studies, being 3 g/d for all but one study, which used 4 g/d (36), and any future studies should consider a minimum dose of 3g/d. Regarding the combined preparations of HMB that have been used (HMB/ARG/GLN, HMB in ONS), although such an approach may be potentially beneficial, these distract from our understanding of the mechanism of action of HMB alone on skeletal muscle anabolism and catabolism as protein and vitamin D impact muscle mass gain and muscle strength but through differing mechanisms (12, 20, 21, 53).

Strengths and limitations

This systematic review and meta-analysis has several strengths related to the robust methodological approach. The protocol was pre-registered on PROSPERO and all eligibility screening, data extraction and risk of bias assessment was undertaken independently in duplicate, with a third person available for arbitration if required, thus limiting the potential for error and bias.

The limitations of this systematic review relate to the quality of the design and reporting of the included studies. First, although we contacted seven authors to obtain missing data, only one responded despite several follow-up attempts, this

combined with inconsistent reporting of data across studies, required some standard deviations to be imputed (28). No study was considered at low risk of bias across all domains. Most prominent was the lack of blinding and compliance with the intervention was a significant issue in several of the studies.

The studies included utilised varying measures of body composition analysis including dual energy X-ray absorptiometry, air displacement plethysmography (Bod Pod), CT and BIA, with some studies using more than one method. It is possible that HMB may influence different compartments of FFM and lean mass differently and therefore these techniques may provide different results depending upon the compartments measured and the choice of measurement techniques for strength and physical function may influence results (e.g. handgrip vs. leg-extensor strength). Thus, techniques to measure both muscle mass and strength should be carefully considered in future trials of HMB.

Although we also included surrogate markers of muscle wasting as an outcome measure, these results are more difficult to interpret. The rationale for BUN and UUN as surrogate markers for muscle wasting is controversial and measuring nitrogen balance in the critically ill population has several limitations (54). Future studies should investigate muscle protein turnover using stable isotopes to ensure the reporting of more precise indications of the effect of HMB on muscle wasting.

Conclusion

Investigating interventions that reduce skeletal muscle wasting and maintain or improve muscle mass are a clinical priority. This systematic review and meta-analysis of RCTs found that HMB alone, and supplements containing HMB, improve muscle mass and muscle strength in a variety of clinical groups, although the effect

size was small to moderate. Furthermore, sub-group analysis revealed strong evidence to support the use of HMB to increase muscle strength. However, given the bias associated with many of the included studies, further, high quality RCTs should be undertaken with greater methodological rigor.

Conflicts of interest

DEB reports receiving advisory board fees, speaker fees and conference attendance support from Nutricia, Nestle Nutrition, BBraun, Baxter healthcare, Fresenius Kabi and Abbott Nutrition. LW reports conference attendance support from Fresenius Kabi. AL, ED, SDR, NH, BC and KW report no conflicts of interest.

Statement of authorship

DEB, KW, BC, NH, SDRH designed the research; DB, AL, ED, LW, KW conducted research; DB, ED analyzed data; DB drafted the manuscript; DB had primary responsibility for final content. All authors interpreted the data and contributed to, read and approved the final manuscript.

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Table 1 - Inclusion and exclusion criteria used to evaluate studies for the systematic review

	Inclusion criteria	Exclusion criteria
Study design	Randomized controlled trial	Non-randomized controlled trial
Population	Adult (≥ 18 years) with a primary clinical diagnosis (eg. chronic obstructive pulmonary disease, cancer, malnutrition) In- or outpatient setting	Children, athletes, healthy elderly, animals
Intervention	Minimum 1.5 g/d β-hydroxy-β-methylbutyrate either alone or in combination with other nutrients of any duration	<1.5 g/d β-hydroxy-β-methylbutyrate
Comparator	Placebo or usual care	Nil
Outcome measure	Muscle mass (measured by any means), body composition (measured by any means), strength, physical function	Specified outcome measures not investigated (e.g. clinical indices only)

Table 2 - Study characteristics of included trials.

Study	Patient population	Age (years)	BMI (kg/m^2)	Intervention	Control	Duration of Intervention	Outcome and outcome relevant to review	Other outcomes
Hsieh (35)	Mechanically ventilated COPD	I: 78.8 (9.7) C: 78.3 (7.4)	I: 21.1 (3.72) C: 18.69 (3.33)	HMB 3 g (2 x 1.5.g doses / d)	Usual care	7 days	Inflammation (CRP) Protein metabolism (BUN) Body composition (Body weight)	Pulmonary function
Hsieh (36)	Tube fed older people	I: 72.5 (11.8) C: 70.8 (9.8)	I: 19.2 (4) C: 21.6 (3.1)	HMB 4 g (2 x 2 g doses / d)	Usual care	14 days plus a subgroup for 28 days	Body composition (thorax, waist, hip, calf circumference, MAMC, TSF) Protein metabolism (BUN)	Nil
Fitschen (39)	Maintenance haemodialysis	I: 57 (8) C: 53 (13)	I: 31.9 (7) C: 30.8 (6.4)	HMB 3 g (3 x 1 g doses / d)	Non-nutritive placebo	6 months	Body composition (DXA, body weight) Muscle strength (knee extension and flexion isokinetic muscle torque) Physical function (shuttle walk, sit-to-stand, up-and-go)	Nil
Clark (26)	HIV	I: 40.9 (1.4)[3,6] C: 40.2 (1.3)[3,6]	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Maltodextrin	8 weeks	Body composition (body weight, forearm, upper arm and thigh circumference, TSF, Bod Pod, CT thigh)	Blood chemistry (liver function tests, blood lipids, hematologic parameters, HIV viral load, BUN)

May (27)	Cancer Cachexia (stage IV, advanced solid tumours)	I: 66 (2.3)[3,6] C:66 (2.1)[3.6]	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Iso-nitrogenous control with non-essential amino acids (L-alanine 11 g, L-glutamic acid 1.75 g, L-glycine 6.10 g, L-serine 4.22 g)	24 weeks	Body composition (body weight, BIA, Bod Pod)	Quality of life (SF-36) Dietary Intake
Marcora (40)	Rheumatoid arthritis	I: 54 (10) C: 57 (8)	I: 25.2 (4.1) C: 27.2 (4.8)	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Iso-nitrogenous control with non-essential amino acids (L-alanine 11 g, L-glutamic acid 1.75 g, L-glycine 6.10 g, L-serine 4.22 g)	12 weeks	Body composition (DXA, BIA) Physical function (sit-to-stand, modified HAQ, advanced ADLs scale, habitual physical activity)	Dietary intake Disease activity index
Kuhls (25)	Mechanically ventilated trauma	I: 36 (3.2)[6]; 41 (3.2)[6] C: 37 (3.3)[6]	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d) and 3 g HMB (2 x 1.5 g doses / d)[1]	Iso-nitrogenous control with non-essential amino acids (30.6 g of hydrolyzed gelatin, 7.8 g L-alanine, 4.2 g L-glycine, 3.0 g L-serine, and 1.2 g L-glutamic acid)	14 days	Protein metabolism (nitrogen balance, 3-MH excretion) Inflammation (SIRS score)	Pre-albumin
Berk (41)	Cancer Cachexia	I: 67 (23,91)[4] C: 65 (35,90)[4]	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-	Iso-nitrogenous control with non-essential amino acids (30.52 g gelatin, L-alanine 7.72 g,	8 weeks	Body composition (body weight, BIA, upper arm, forearm, chest, hip and thigh circumference, 7 site	Fatigue (Schwartz fatigue score) Quality of life (Spritzer quality of life index)

				glutamine 7 g doses / d)	L-glutamic acid 1.23 g, L-glycine 4.28 g, L-serine 2.96 g)		skinfold thickness, Bod Pod)	
Clements (45)	Gastric bypass	I: 47.9 (9.6) C: 46 (7.5)	I: 42.9 (4.1) C: 43.6 (4.2)	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Usual care	8 weeks	Body composition (body weight, DXA) Resting metabolic rate	Nil
Olveira (37)	Bronchiectasis	I: 58.4 (12.9) C: 53.7 (13.1)	I: 25.9 (3.4) C: 27.3 (5.8)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	24 weeks	Body composition (body weight, BIA phase-angle; DXA, MAMC) Strength (handgrip dynamometry)	Quality of life Dietary intake Myostatin, Somatomedin C, Insulin
Ekinci (42)	Older females with hip fracture	I: 82.19 (7.28) C: 83.07 (7.08)	I: 21.83 (2.11) C: 22.25 (2.7)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	30 days	Body composition (body weight, calf and arm circumference, TSF, MAMC) Muscle strength (handgrip dynamometry)	Immobilisation period Wound healing CRP
Deutz (43)	Hospitalised, older people with malnutrition[2]	I: 77.7 (8.2) C:78.1 (8.6)	I: 24.3 (5.2) C: 23.9 (5)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D	Placebo containing 48 kcal, 12 g CHO, 10mg Vitamin C	90 days	Post-discharge incidence of death or non-elective readmission Length of stay	SGA class Vitamin D level ADLs

				(ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)			Body composition (Body weight)	
Cramer (31)	Older people with malnutrition and sarcopenia	I: 77 (71,81)[5] C: 77 (71,81)[5]	I: 25 (23,29)[5] C: 26 (24,29)[5]	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	ONS containing 330 kcal, 14 g pro, 147 IU Vitamin D_3	24 weeks	Body composition (body weight, DXA) Strength (grip strength, leg strength) Physical function (gait speed)	Nil
Nishizaki (38)	Older people following knee arthroplasty	I: 71.1 (NR) C: 69.8 (NR)	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Orange juice containing 113 kcal and 140 mg pro	5 days before and 28 days after surgery	Body composition (body weight, CT of RFcsa) Strength (knee extensor)	Nil
Malafarina (44)	Older people with hip fracture	I: 85.7 (6.5) C: 84.7 (6.3)	I: 24.9 (4.4) C: 26 (5.4)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	Duration of rehabilitation unit stay	Body composition (body weight, BIA) Strength (hand grip dynamometry) Physical function (gait speed)	Plasma Vitamin D

[1]30.6 g of hydrolyzed gelatin, 7.8 g L-alanine, 4.2 g L-glycine, 3.0 g L-serine, and 1.2 g L-glutamic acid added to HMB alone supplement to make isonitrogenous.
[2]Patients with congestive heart failure, acute myocardial infarction, pneumonia or chronic obstructive pulmonary disease
[3]Completed patients; [4]Median (range); [5]Median (interquartile range); [6]Mean (SE)
ADLs = activities of daily living; BF = breakfast; BIA = bioelectrical impedance analysis; BMI = body mass index; BUN = blood urea nitrogen; C = control; CHO = carbohydrate; CRP = C-Reactive Protein; CT = computed tomography; D = dinner; HAQ = health assessment questionnaire; HIV =

Human Immunodeficiency Virus; I = intervention; IU = international units; L = lunch; MAMC = mid-arm muscle circumference; NR = not reported; ONS = oral nutrition supplement; Pro = protein; RFcsa = rectus femoris cross sectional area; SGA = subjective global assessment; SF-36 = short-form 36; TSF = triceps skin fold; 3-MH = 3 methyl-histidine

Figure titles and legends

Figure 1: Flow diagram of study selection process

Figure 2: Risk of bias summary for all studies and outcomes

Figure 3: Forest plot for the effect of HMB or supplements containing HMB on change in muscle mass

Forest plot of a random effects meta-analysis of nine studies for change in muscle mass. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

Figure 4: Forest plot showing the effect of HMB or supplements containing HMB on change in body weight.

Forest plot of a random effects meta-analysis of twelve studies for change in body weight. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

Figure 5: Forest plot showing the effect of HMB or supplements containing HMB on absolute strength.

Forest plot of a random effects meta-analysis of six studies for absolute strength. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination

with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

Figure 1 -

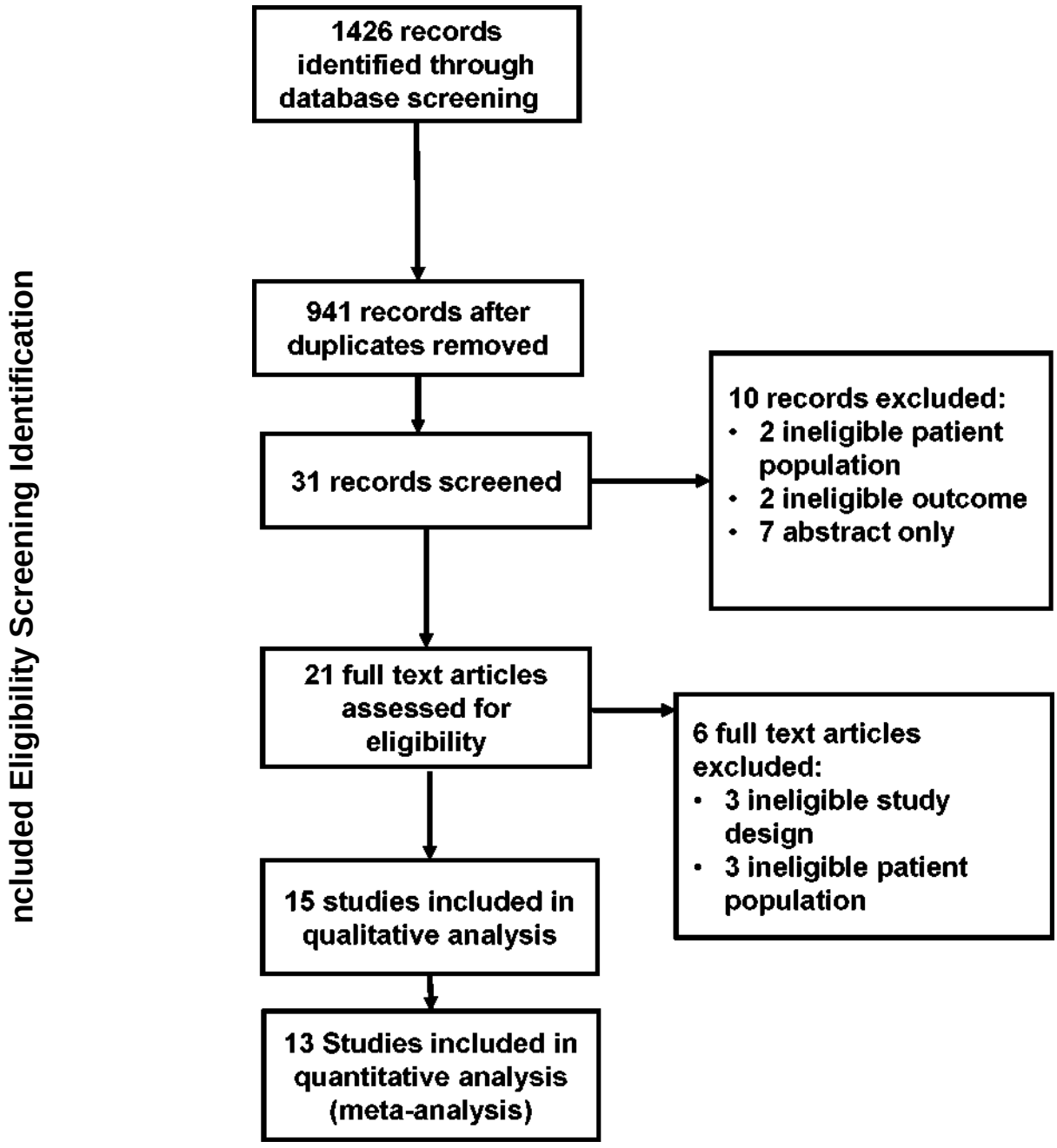
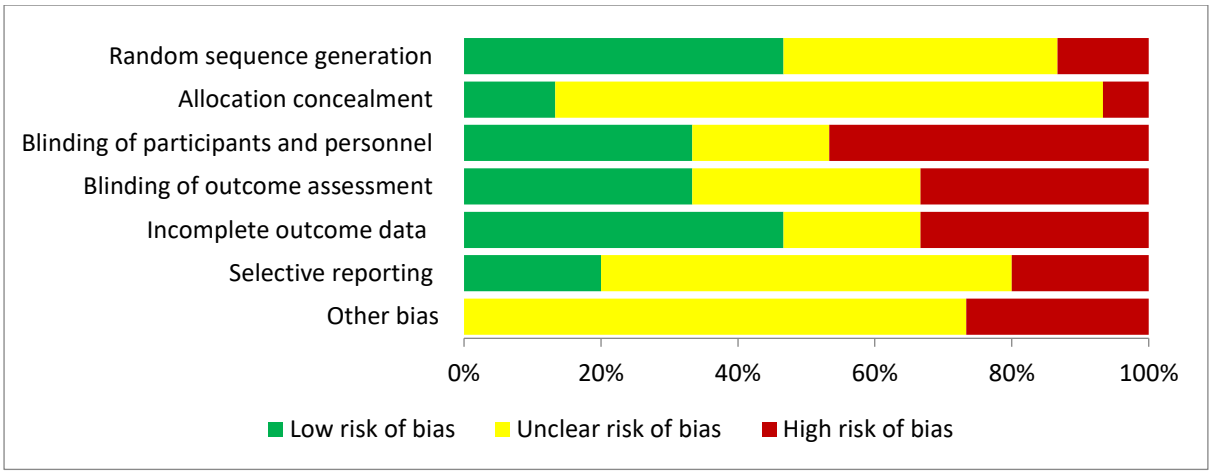

ncluded Eligibility Screening Identification
1426 records identified through database screening
941 records after duplicates removed
31 records screened
10 records excluded:
• 2 ineligible patient population
• 2 ineligible outcome
• 7 abstract only
21 full text articles assessed for eligibility
6 full text articles excluded:
• 3 ineligible study design
• 3 ineligible patient population
15 studies included in qualitative analysis
13 Studies included in quantitative analysis (meta-analysis)

Figure 2 -

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Hsieh 2006	−	−	−	?	?	?	?
Hsieh 2010	?	?	−	?	?	?	?
Fitschen 2016	?	?	?	?	−	+	?
Clark 2000	+	?	?	?	+	?	?
May 2002	+	?	+	+	−	?	?
Marcora 2005	+	+	+	+	+	?	−
Kuhls 2007	+	?	+	+	−	+	?
Berk 2008	?	?	?	?	−	?	−
Clements 2011	?	?	−	−	+	?	?
Olivera 2015	+	?	−	−	+	−	?
Ekinci 2016	−	?	−	−	+	?	−
Deutz 2017	+	+	+	+	+	−	−
Cramer 2016	?	?	+	+	−	+	?
Malafarina 2017	+	?	−	−	?	−	?
Nishizaki 2015	?	?	−	−	+	?	?



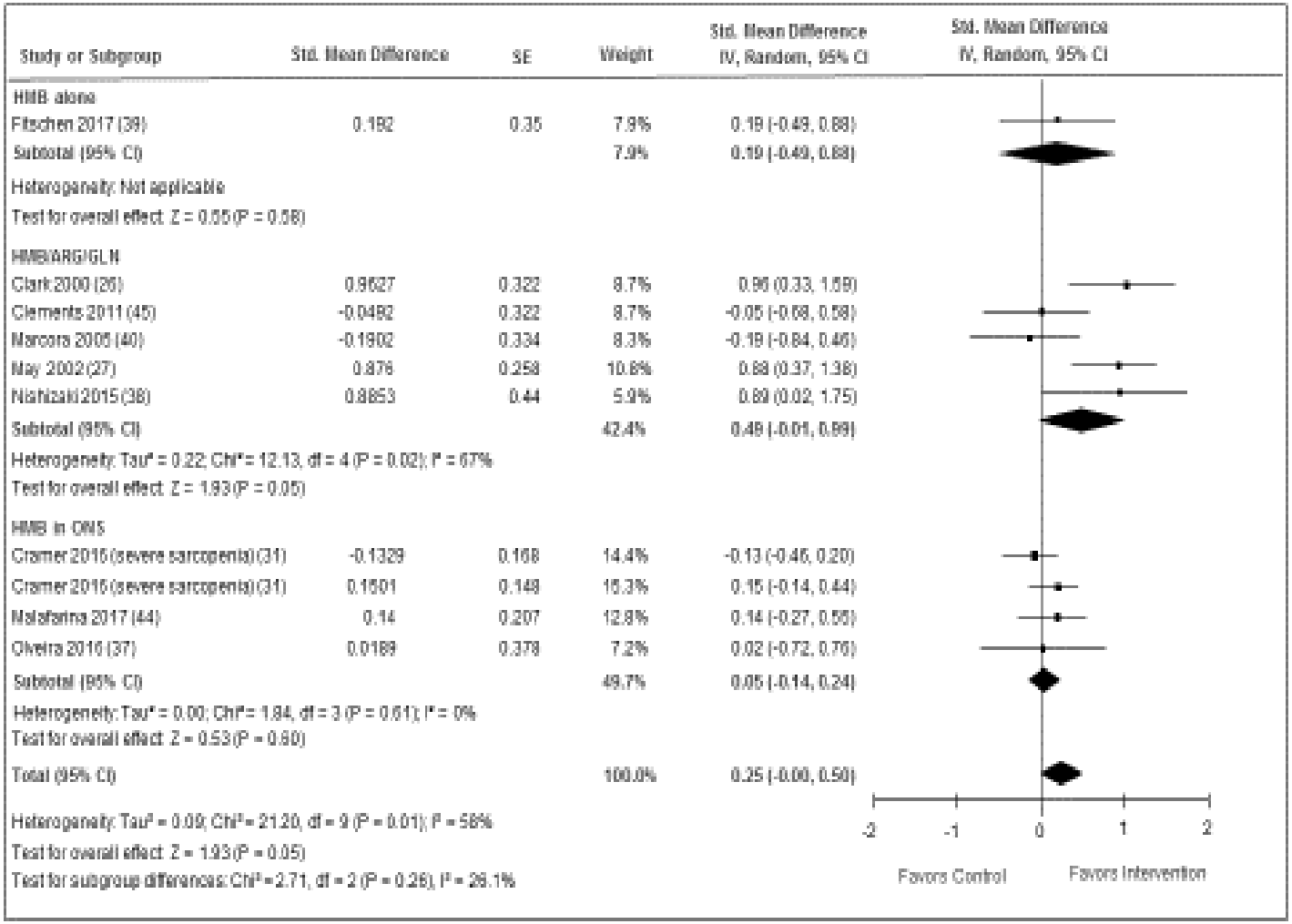

Study or Subgroup
Std. Mean Difference
SE
Weight
Std. Mean Difference IV, Random, 95% CI
Std. Mean Difference IV, Random, 95% CI
HMB alone
Fitschen 2017 (39) 0.192 0.35 7.9% 0.19 [-0.49, 0.88]
Subtotal (95% CI) 7.9% 0.19 [-0.49, 0.88]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.55 (P = 0.58)
HMB/ARG/GLN
Clark 2000 (26) 0.9627 0.322 8.7% 0.96 [0.33, 1.59]
Clements 2011 (45) -0.0492 0.322 8.7% -0.05 [-0.68, 0.58]
Marcora 2005 (40) -0.1902 0.334 8.3% -0.19 [-0.84, 0.46]
May 2002 (27) 0.876 0.258 10.8% 0.88 [0.37, 1.38]
Nishizaki 2015 (38) 0.8853 0.44 5.9% 0.89 [0.02, 1.75]
Subtotal (95% CI) 42.4% 0.48 [-0.01, 0.99]
Heterogeneity: Tau² = 0.22; Chi² = 12.13, df = 4 (P = 0.02); I² = 67%
Test for overall effect: Z = 1.93 (P = 0.05)
HMB in ONS
Cramer 2015 (severe sarcopenia) (31) -0.1329 0.168 14.4% -0.13 [-0.46, 0.20]
Cramer 2015 (severe sarcopenia) (31) 0.1501 0.149 15.3% 0.15 [-0.14, 0.44]
Malafarina 2017 (44) 0.14 0.207 12.8% 0.14 [-0.27, 0.55]
Oliveira 2016 (37) 0.0189 0.378 7.2% 0.02 [-0.72, 0.76]
Subtotal (95% CI) 49.7% 0.05 [-0.14, 0.24]
Heterogeneity: Tau² = 0.00; Chi² = 1.84, df = 3 (P = 0.61); I² = 0%
Test for overall effect: Z = 0.53 (P = 0.60)
Total (95% CI) 100.0% 0.25 [-0.00, 0.50]
Heterogeneity: Tau² = 0.09; Chi² = 21.20, df = 9 (P = 0.01); I² = 58%
Test for overall effect: Z = 1.93 (P = 0.05)
Test for subgroup differences: Chi² = 2.71, df = 2 (P = 0.26), I² = 26.1%
-2
-1
0
1
2
Favors Control
Favors Intervention

Figure 3 -

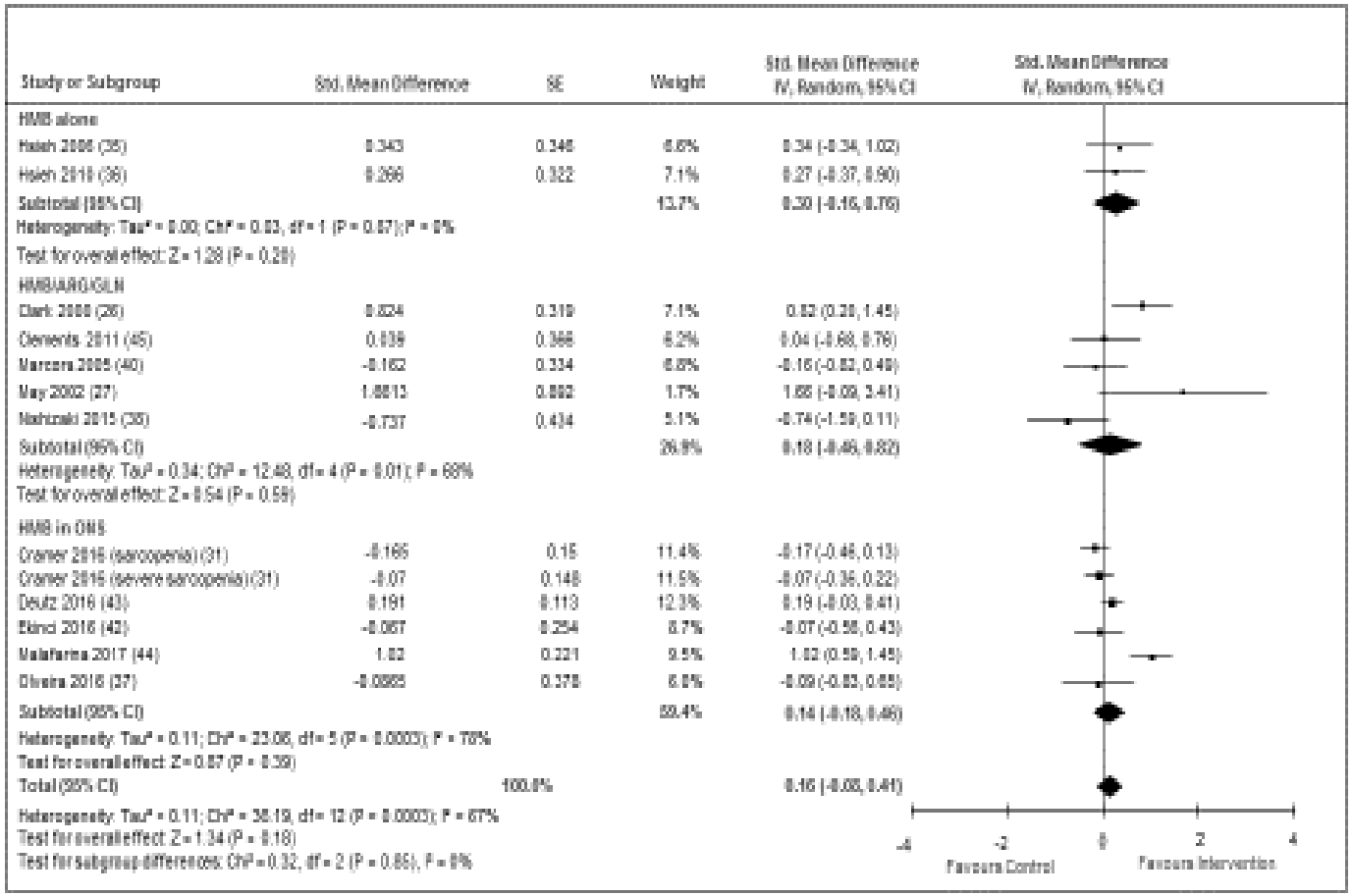

Study or Subgroup
Std. Mean Difference
SE
Weight
Std. Mean Difference IV, Random, 95% CI
Std. Mean Difference IV, Random, 95% CI
HMB alone
Hsieh 2006 (35) 0.343 0.346 6.6% 0.34 [-0.34, 1.02]
Hsieh 2010 (36) 0.266 0.322 7.1% 0.27 [-0.37, 0.90]
Subtotal (95% CI) 13.7% 0.30 [-0.16, 0.76]
Heterogeneity: Tau² = 0.00; Chi² = 0.02, df = 1 (P = 0.87); I² = 0%
Test for overall effect: Z = 1.28 (P = 0.20)
HMB/ARG/GLN
Clark 2000 (26) 0.824 0.319 7.1% 0.82 [0.20, 1.45]
Clements 2011 (45) 0.039 0.366 6.2% 0.04 [-0.68, 0.76]
Marcora 2005 (40) -0.162 0.334 6.8% -0.16 [-0.82, 0.49]
May 2002 (27) 1.6813 0.892 1.7% 1.68 [-0.09, 3.41]
Nakaki 2015 (30) -0.737 0.434 5.1% -0.74 [-1.59, 0.11]
Subtotal (95% CI) 26.8% 0.18 [-0.46, 0.82]
Heterogeneity: Tau² = 0.34; Chi² = 12.48, df = 4 (P = 0.01); I² = 68%
Test for overall effect: Z = 0.54 (P = 0.59)
HMB in ONS
Cramer 2016 (sarcopenia) (31) -0.165 0.15 11.4% -0.17 [-0.46, 0.13]
Cramer 2016 (severe sarcopenia) (31) -0.07 0.148 11.5% -0.07 [-0.36, 0.22]
Deutz 2016 (43) 0.191 0.113 12.3% 0.19 [-0.03, 0.41]
Ekinci 2016 (42) -0.067 0.254 9.7% -0.07 [-0.56, 0.43]
Malafarina 2017 (44) 1.02 0.221 9.5% 1.02 [0.59, 1.45]
Oliveira 2016 (37) -0.0885 0.378 8.8% -0.09 [-0.83, 0.65]
Subtotal (95% CI) 59.4% 0.14 [-0.18, 0.46]
Heterogeneity: Tau² = 0.11; Chi² = 23.06, df = 5 (P = 0.0003); I² = 78%
Test for overall effect: Z = 0.87 (P = 0.39)
Total (95% CI) 100.0% 0.16 [-0.08, 0.41]
Heterogeneity: Tau² = 0.11; Chi² = 36.19, df = 12 (P = 0.0003); I² = 67%
Test for overall effect: Z = 1.34 (P = 0.18)
Test for subgroup differences: Chi² = 0.32, df = 2 (P = 0.85), I² = 0%
-4
-2
0
2
4
Favours Control
Favours Intervention

Figure 4 -

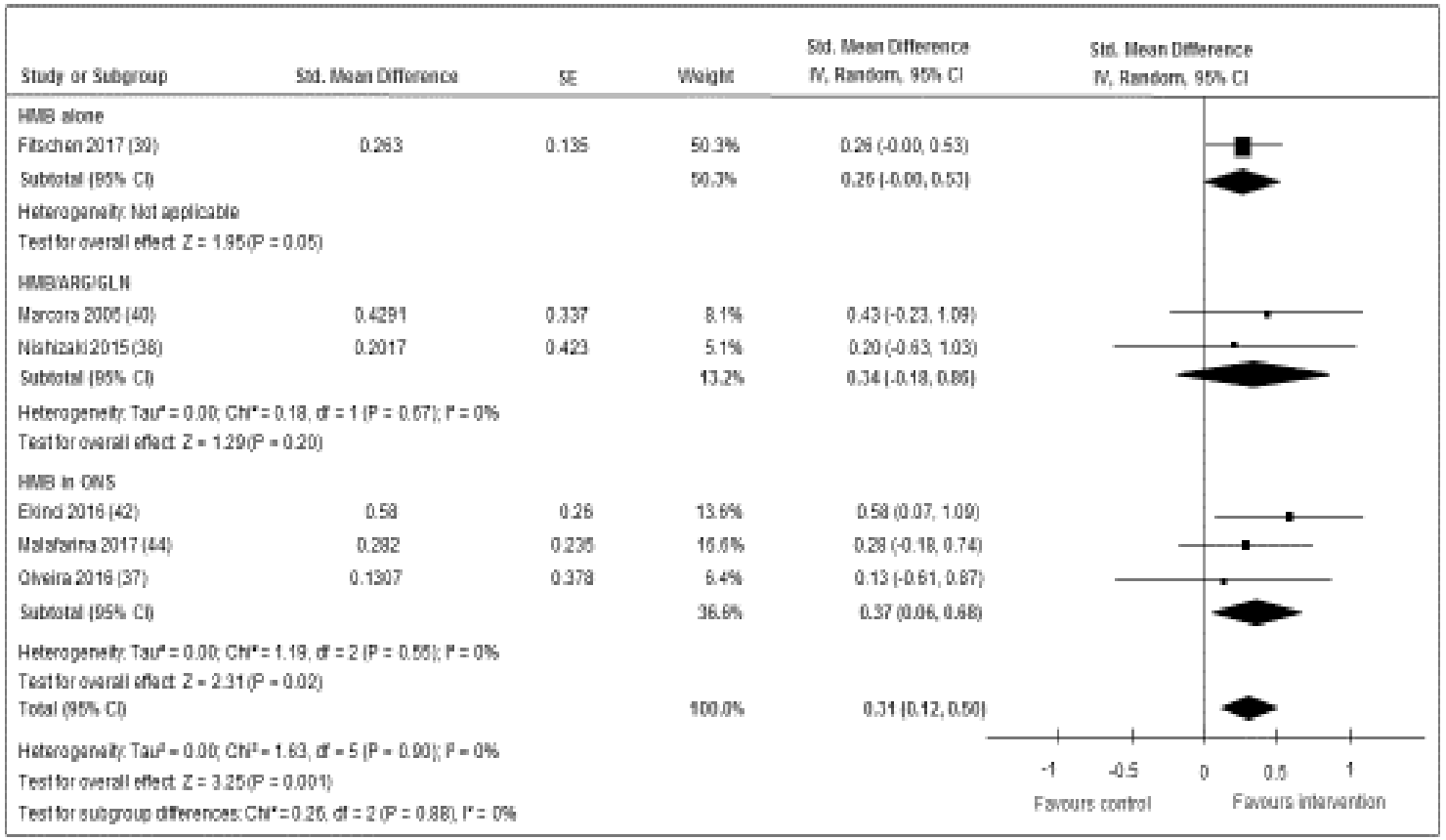

Study or Subgroup
Std. Mean Difference
SE
Weight
Std. Mean Difference IV, Random, 95% CI
Std. Mean Difference IV, Random, 95% CI
HMB alone
Fitschen 2017 (39) 0.263 0.135 50.3% 0.26 (-0.00, 0.53)
Subtotal (95% CI) 50.3% 0.26 (-0.00, 0.53)
Heterogeneity: Not applicable
Test for overall effect: Z = 1.95 (P = 0.05)
HMB/ARG/GLN
Marcora 2008 (40) 0.4291 0.337 8.1% 0.43 (-0.23, 1.09)
Nishizaki 2015 (38) 0.2017 0.423 5.1% 0.20 (-0.63, 1.03)
Subtotal (95% CI) 13.2% 0.34 (-0.18, 0.86)
Heterogeneity: Tau² = 0.00; Chi² = 0.18, df = 1 (P = 0.67); I² = 0%
Test for overall effect: Z = 1.29 (P = 0.20)
HMB in ONS
Ekind 2016 (42) 0.58 0.26 13.6% 0.58 (0.07, 1.09)
Malafarina 2017 (44) 0.282 0.235 16.6% 0.28 (-0.18, 0.74)
Oliveira 2016 (37) 0.1307 0.378 6.4% 0.13 (-0.61, 0.87)
Subtotal (95% CI) 36.6% 0.37 (0.06, 0.68)
Heterogeneity: Tau² = 0.00; Chi² = 1.19, df = 2 (P = 0.55); I² = 0%
Test for overall effect: Z = 2.31 (P = 0.02)
Total (95% CI) 100.0% 0.31 (0.12, 0.50)
Heterogeneity: Tau² = 0.00; Chi² = 1.63, df = 5 (P = 0.90); I² = 0%
Test for overall effect: Z = 3.25 (P = 0.001)
Test for subgroup differences: Chi² = 0.25, df = 2 (P = 0.88), I² = 0%
-1
-0.5
0
0.5
1
Favours control
Favours intervention

Figure 5 -

Supplemental Methods

Example search strategy

Database: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

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1 HMB.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (2626)

2 beta-hydroxy-beta-methylbutyrate.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (262)

3 'beta hydroxy beta methylbutyrate'.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (262)

4 1 or 2 or 3 (2674)

5 muscle wasting.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (4399)

6 muscle loss.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1448)

7 muscle mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (14504)

8 skeletal muscle.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (107133)

9 sarcopenia.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (5143)

10 cache*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (11284)

11 physical fitness.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (31143)

12 physical function.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (11321)

13 fat free mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (6874)

14 fat mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (18679)

15 muscle strength.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (29829)

16 grip strength.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (9374)

17 strength.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (278916)

18 body composition.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (53932)

19 lean body mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (7378)

20 maln*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (47521)

21 exp Muscle, Skeletal/ or exp Cachexia/ or exp Muscular Atrophy/ or exp Muscles/ (715992)

22 exp Body Composition/ or exp Sarcopenia/ (53752)

23 exp Muscle Strength/ or exp Body Weight/ (475694)

24 exp Hand Strength/ or exp Muscle Strength Dynamometer/ (14388)

25 exp Physical Fitness/ (27735)

26 exp "Activities of Daily Living"/ (66805)

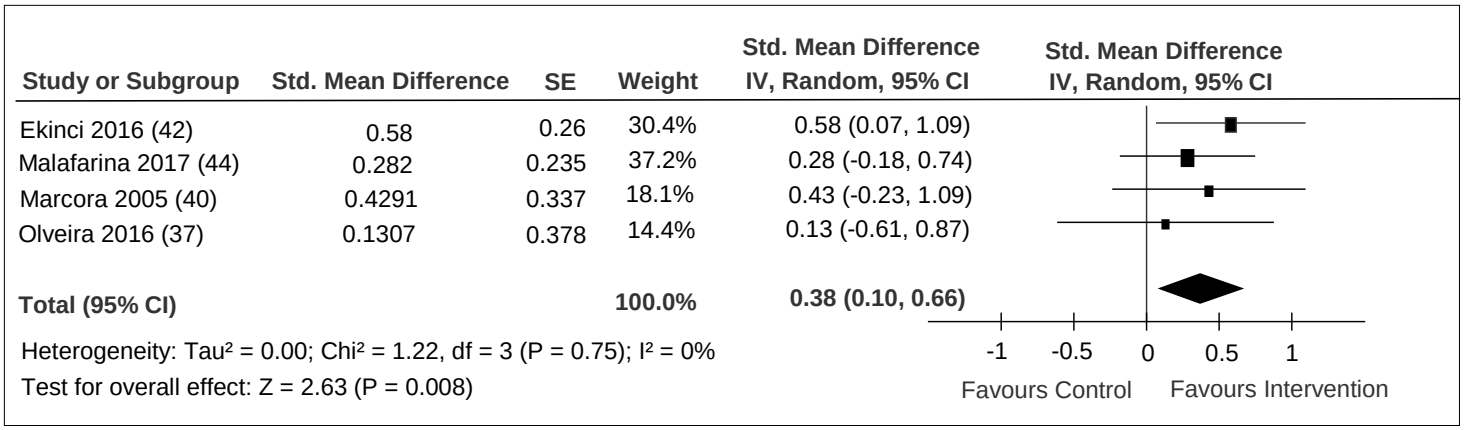
27 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 (1576685)

28 4 and 27 (311)

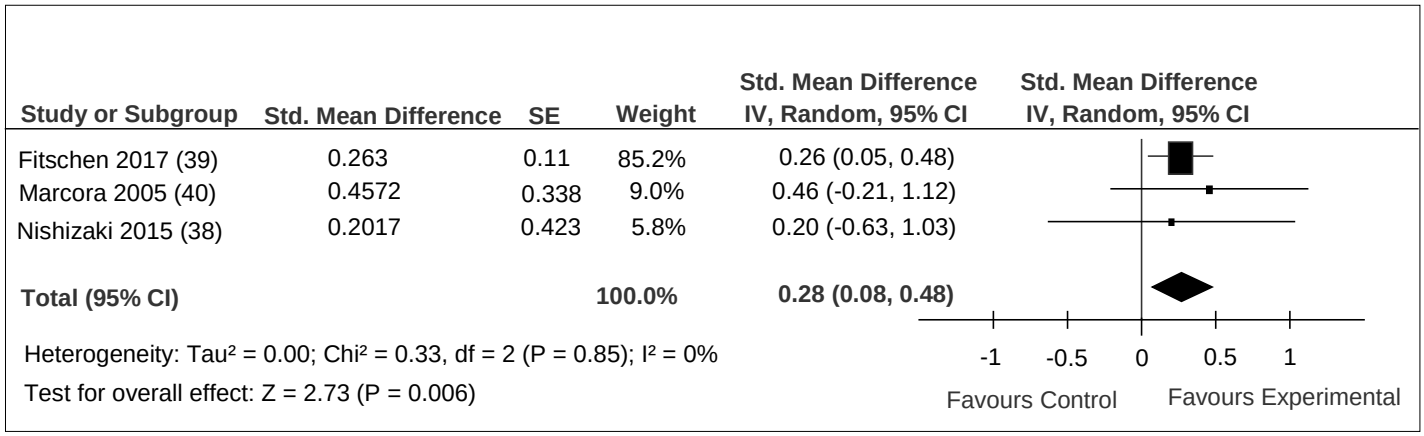
29 exp animals/ not humans.sh. (4860425)

30 28 not 29 (236)

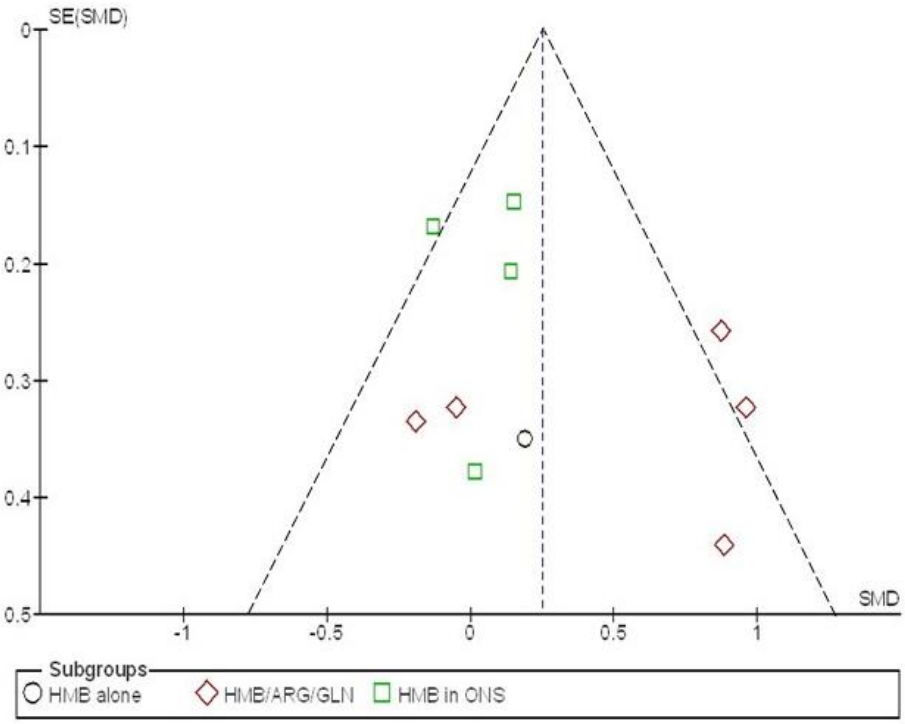
Supplemental Results



Supplemental Figure 1: Forest plot of a random effects meta-analysis of four studies for handgrip strength. Results are presented as standardized mean difference with 95% confidence intervals.

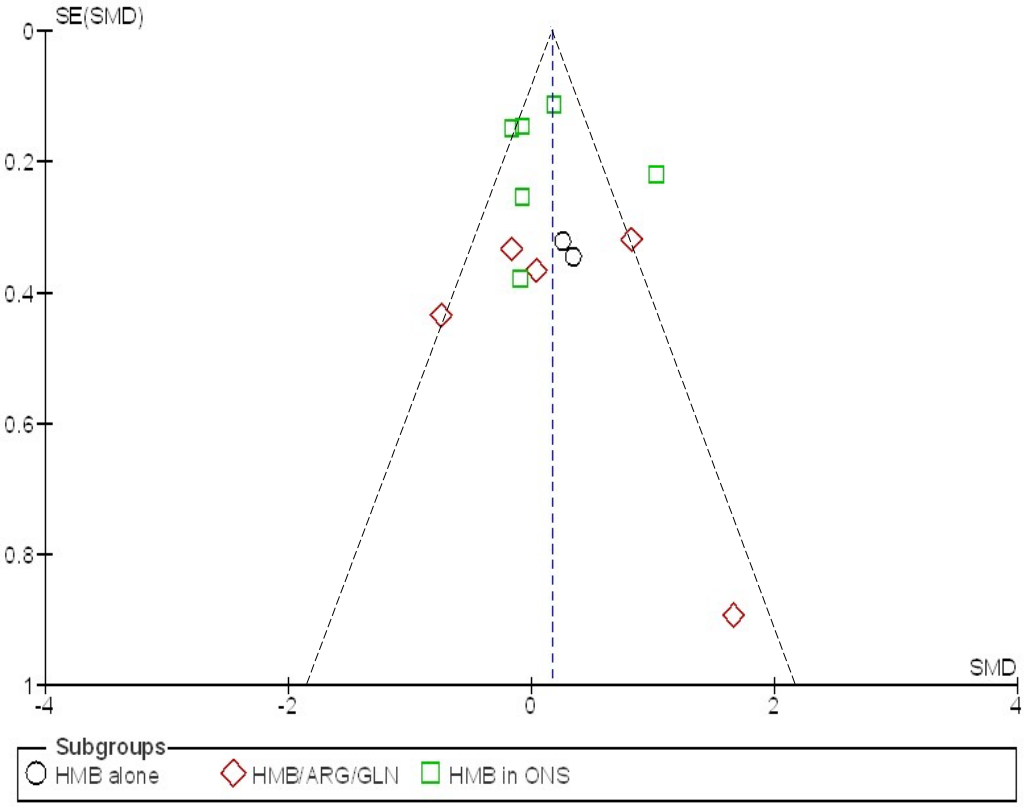


Supplemental Figure 2: Forest plot of a random effects model of three studies for leg strength. Results are presented as standardized mean difference with 95% confidence intervals.



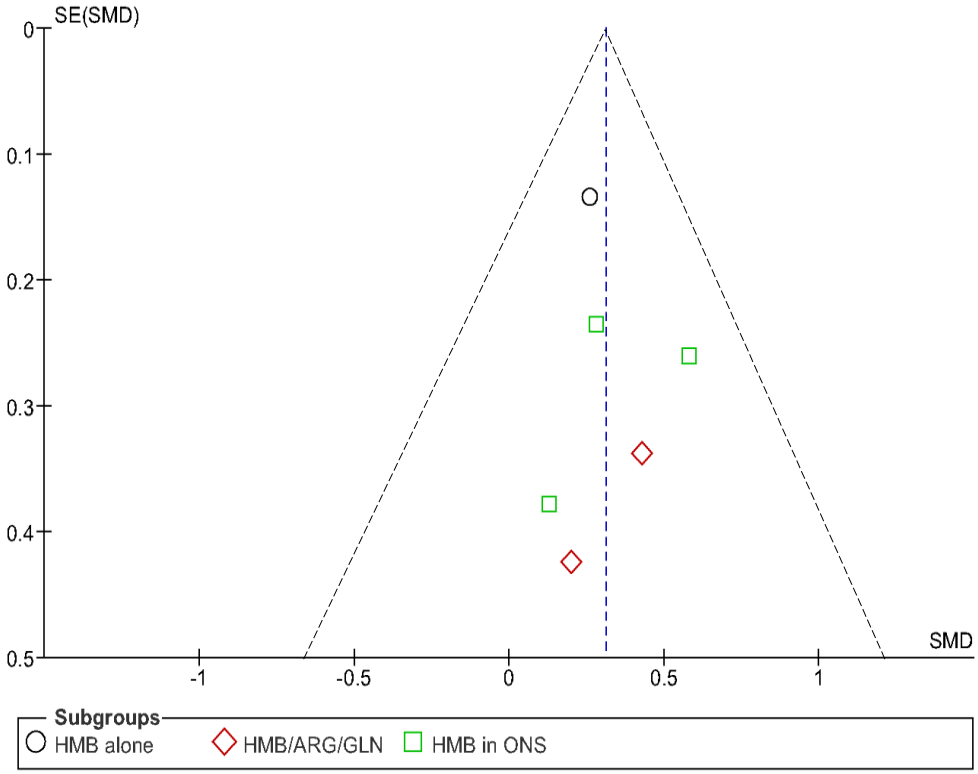
Supplemental Figure 3 – Funnel plot of change in muscle mass.

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of change in muscle mass is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the 9 included trials. Visual inspection of the funnel plot does not reveal any substantial asymmetry and hence publication bias.



Supplemental Figure 4 – Funnel plot of change in body weight.

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of change in body weight is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the 12 included trials.



Supplemental Figure 5 – Funnel plot of change in absolute strength.

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of absolute strength is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the six included trials.